O'Bryen, Barbara

From: Chan, Christina
Sent: Friday, March 23, 2001 9:04 AM
To: Bui, Phuong; O'Bryen, Barbara
Subject: RE: 09/410835 sequence search

Importance: High

Please rush. Thanks Chris

Chris Chan
TC 1600 New Hire Training Coordinator and SPE, 1644
CM 1, Room 9B19
308-3973

-----Original Message-----
From: Bui, Phuong
Sent: Thursday, March 22, 2001 7:39 PM
To: Chan, Christina
Subject: 09/410835 sequence search

Chris,

Please forward the following search to **Barb O'Bryen** as a rush. 2-month amendment. Thanks. P.

Applic. No. 09/410835
Phuong Bui
AU 1638
CM1, 9A09; Mailbox 9E12
305-1996

Please search 15-mers of SEQ ID NO. 4. Since this case has priority back to 1992, please list citations for the top 200 hits.

Please include an interference search of SEQ ID NO:4.

Thank you! Phuong.

POINT OF CONTACT:
BARB O'BRYEN
TECH. INFORMATION SPECIALIST
STIC CM1 12C14 308-4291

BOB
3-27-01

L15 ANSWER 188 OF 209 MEDLINE
AN 87246068 MEDLINE
DN 87246068 PubMed ID: 3109864
TI A genetically engineered P450 monooxygenase: construction of the functional fused enzyme between rat cytochrome P450c and NADPH-cytochrome P450 reductase.
AU Murakami H; Yabusaki Y; Sakaki T; Shibata M; Ohkawa H
SO DNA, (1987 Jun) 6 (3) 189-97.
Journal code: EAW; 8302432. ISSN: 0198-0238.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198708
ED Entered STN: 19900305
Last Updated on STN: 19970203
Entered Medline: 19870810
AB A hybrid cDNA encoding a fused enzyme consisting of rat cytochrome P450c and rat NADPH-cytochrome P450 reductase was constructed by combining the cytochrome P450c cDNA with the cDNA fragment encoding the protease-solubilized moiety of the NADPH-cytochrome P450 reductase. The hybrid cDNA was inserted between the yeast alcohol dehydrogenase I promoter and terminator of the expression vector pAAH5 to yield expression plasmid pAMP19. Saccharomyces cerevisiae AH22 cells transformed with the expression plasmid pAMP19 produced a **130-kD** protein reactive with both anti-cytochrome P450c Ig and antireductase Ig. The yeast cells containing the fused enzyme exhibited about four times higher monooxygenase activity toward 7-ethoxycoumarin than those containing rat cytochrome P450c alone. The fused enzyme was purified from the yeast microsomal fraction by sequential chromatography with DEAE-cellulose and 2',5'-ADP Sepharose 4B columns. The preparation had an apparent molecular weight of **130 kD** and the same sequence of the 10 amino-terminal amino acids as that of rat cytochrome P450c. Spectral properties of the fused enzyme indicated the presence of a protoheme, flavin adenine dinucleotide, and flavin mononucleotide in the molecule. The reaction mechanism of the fused enzyme followed first-order kinetics. These results clearly indicate that the fused enzyme is a new self-catalytic P450 monooxygenase. Trypsin treatment of yeast microsomes containing the fused enzyme suggested that the P450 moiety is embedded in the microsomal membrane with the reductase moiety lying on the cytoplasmic

L15 ANSWER 208 OF 209 MEDLINE
AN 85150284 MEDLINE
DN 85150284 PubMed ID: 6241481
TI Cloning and expression in Escherichia coli of the TL-DNA gene 4 of Agrobacterium tumefaciens under the control of the PR promoter of bacteriophage lambda.
AU Sibold L; Guiso N; de Beuckeleer M; Van Montagu M
SO BIOCHIMIE, (1984 Jul-Aug) 66 (7-8) 547-56.
Journal code: A14; 1264604. ISSN: 0300-9084.
CY France
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198504
ED Entered STN: 19900320
Last Updated on STN: 19900320
Entered Medline: 19850426
AB A plasmid was constructed that directs expression of the TL-DNA gene 4 protein in E. coli. The different steps of the construction were as follows: i) a region of gene 4 encoding the amino-terminal portion of the protein was fused in frame to DNA encoding an enzymatically active carboxy-terminal fragment of beta-galactosidase. The hybrid gene was poorly expressed from the upstream lambda PL promoter carried by the vector. ii) in order to generate an efficient procaryotic ribosome binding site, a DNA fragment carrying the lambda PR promoter with the nearby Shine-Dalgarno (SD) sequence of gene cro was placed in front of the gene 4-lacZ fusion. A **recombinant** plasmid, termed pGV793, that expressed efficiently a fused protein 4-beta-galactosidase was identified among the Lac+ clones. DNA sequencing analysis showed that pGV793 carried a hybrid ribosome binding site composed of the cro SD sequence, a five bp sequence and the ATG codon of gene 4. Plasmid pGV793 directed the synthesis of three polypeptides of molecular weight **132 Kd**, 126 Kd and 122 Kd that carried beta-galactosidase antigenic determinants. The largest polypeptide had the expected size for the hybrid protein. The fusion proteins which accounted for about 0.5% of the total cellular proteins were purified by immunoadsorption using anti-beta-galactosidase antiserum. iii) the complete gene 4 coding sequence was reconstituted, with the lambda PR promoter in place. The resulting pGV822 plasmid expressed a polypeptide whose molecular weight 27 Kd corresponded to the expected size for the gene 4 product. The pI was

6/7/5 (Item 5 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

08404089 BIOSIS NO.: 000094121743
SERUM NEUTRALIZING ANTIBODY RESPONSE TO THE VACUOLATING **CYTOTOXIN** OF **HELICOBACTER**-PYLORI
AUTHOR: COVER T L; CAO P; MURTHY U K; SIPPLE M S; BLASER M J
AUTHOR ADDRESS: DIV. INFECTIOUS DISEASES, A-3310 MEDICAL CENTER NORTH, VANDERBILT UNIVERSITY SCH. MED., NASHVILLE, TENN. 37232-2605.
JOURNAL: J CLIN INVEST 90 (3). 1992. 913-918.
FULL JOURNAL NAME: Journal of Clinical Investigation
CODEN: JCINA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Approximately 50% of **Helicobacter** pylori isolates produce a **cytotoxin** in vitro that induces vacuolation of eukaryotic cells. To determine the in vivo relevance of this phenomenon, we sought to detect **cytotoxin**-neutralizing antibodies in sera from H. pylori-infected persons. As a group, sera from 29 H. pylori-infected patients neutralized the activity of the purified **cytotoxin** to a significantly greater extent then sera from 24 uninfected persons ($P = 0.007$). The **cytotoxin** neutralizing activity in sera from H. pylori-infected persons was mediated predominantly by the purified IgG fraction. Sera from H. pylori-infected persons neutralized the **cytotoxins** produced by multiple H. pylori strains, but failed to neutralize trimethylamine-induced cell vacuolation. Neutralization of **cytotoxin** activity by human or immune rabbit sera was associated with immunoblot IgG recognition of an 87-kD H. pylori protein. Similarly, neutralization of the toxin by sera was associated with IgG recognition of the purified **cytotoxin** in an enzyme-linked immunosorbent assay ($P < 0.0001$). The presence of **cytotoxin**-neutralizing antibodies in sera from H. pylori-infected persons indicates that the **cytotoxins** is synthesized in vivo.

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? ds

Set      Items   Description
S1     1318984   PY=1992
S2       57204   HELICOBACTER OR CAMPYLOBACTER
S3        2672   S1 AND S2
S4        9988   CYTOTOXIN?
S5          41   S3 AND S4
S6          21   RD (unique items)
S7     1325243   PY=1991
S8       39882   PYLORI
S9          26   S7 AND S8 AND S2 AND S4
S10         15   RD (unique items)
```

6/7/6 (Item 6 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

08378894 BIOSIS NO.: 000094109398
EXPRESSION OF 120 KILODALTON PROTEIN AND CYTOTOXICITY IN **HELICOBACTER** -PYLORI
AUTHOR: CRABTREE J E; FIGURA N; TAYLOR J D; BUGNOLI M; ARMELLINI D; TOMPKINS D S
AUTHOR ADDRESS: DEP. MED., ST. JAMES'S UNIV. HOSP., LEEDS LS9 7TF.
JOURNAL: J CLIN PATHOL (LOND) 45 (8). 1992. 733-734.
FULL JOURNAL NAME: Journal of Clinical Pathology (London)
CODEN: JCPAA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Antral biopsy culture supernatants from 14 subjects with chronic gastritis, known to have IgA antibodies to the 120 kilodalton protein, showed positive recognition of this antigen in western blots against a **cytotoxin** positive strain of **Helicobacter** pylori but gave negative reactions with two **cytotoxin** negative strains. Control immunoblots with culture supernatants from 13 non-responders to the protein were all negative. This indicates a direct association between expression of the 120 kilodalton protein in H. pylori strains and cytotoxicity.

DD P

L15 ANSWER 161 OF 209 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 74
AN 1988:242952 BIOSIS
DN BA85:121354
TI HIGH EXPRESSION OF FUNCTIONAL ADENOVIRUS DNA POLYMERASE AND PRECURSOR TERMINAL PROTEIN USING **RECOMBINANT** VACCINIA VIRUS.
AU STUNNENBERG H G; LANGE H; PHILIPSON L; VAN MILTENBURG R T; VAN DER VLIET P
C
CS LAB. PHYSIOLOGICAL CHEMISTRY, STATE UNIV. UTRECHT, VONDELLAAN 24A, 3521 GG
UTRECHT, NETHERLANDS.
SO NUCLEIC ACIDS RES, (1988) 16 (6), 2431-2444.
CODEN: NARHAD. ISSN: 0305-1048.
FS BA; OLD
LA English
AB Initiation of Adenovirus (Ad) DNA replication occurs by a protein-priming mechanism in which the viral precursor terminal protein (pTP) and DNA polymerase (pol) as well as two nuclear DNA-binding proteins from uninfected HeLa cells are required. Biochemical studies on the pTP and DNA
polymerase proteins separately have been hampered due to their low abundance and their presence as a pTP-pol complex in Ad infected cells. We
have constructed a genomic sequence containing the large open reading frame from the Ad5 pol gene to which 9 basepairs from a putative exon were
ligated. When inserted behind a modified late promoter of vaccinia virus the resulting **recombinant** virus produced enzymatically active **140 kDa** Ad DNA polymerase. The same strategy was applied to express the 80 kDa pTP gene in a functional form. Both proteins were overexpressed at least 30-fold compared to extracts from Adenovirus infected cells and, when combined, were fully active for initiation in an in vitro Adenovirus DNA replication system.

L15 ANSWER 172 OF 209 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 78
AN 1988:375442 BIOSIS
DN BA86:59352
TI EIMERIA-ACERVULINA DNA CLONING AND CHARACTERIZATION OF **RECOMBINANT** SPOROZOITE AND MEROZOITE ANTIGENS.
AU JENKINS M C; LILLEHOJ H S; DAME J B
CS UNIV. FLORIDA, DEP. INFECTIOUS DISEASES, GAINESVILLE, FLORIDA 32610.
SO EXP PARASITOL, (1988) 66 (1), 96-107.
CODEN: EXPAAA. ISSN: 0014-4894.
FS BA; OLD
LA English
AB Genes encoding antigens of Eimeria acervulina were cloned from cDNA expression libraries prepared from the sporozoite and merozoite stages in order to examine humoral and cellular immune responses to this protozoan parasite. Two clones expressing surface antigens were characterized by DNA hybridization studies to identify homologous genomic DNA fragments. The proteins they encode were identified by 125I-labeling, immunoblotting, immunofluorescence, and T-cell activation experiments. One, designated cSZ-1, encodes a **130-kDa** .beta.-galactosidase fusion protein which represents a portion of a p240/p160 immunodominant sporozoite surface antigen. Immunofluorescence studies using anti-cSZ-1 sera and live or 1% paraformaldehyde-fixed E. acervulina sporozoites have confirmed this surface locale. Purified cSZ-1 fusion protein, which is not recognized by sera from E. acervulina-infected chickens, induced the activation of immune T lymphocytes in vitro. Another cDNA clone, designated cMZ-8, gives rise to a **150-kDa** fusion protein and encodes a portion of a p250 immunodominant merozoite surface antigen. This was established by immunoblotting of 125I-labeled merzoite proteins with anti-cMZ-8 sera and immunofluorescence staining of live and 1% paraformaldehyde-fixed E. acervulina merozoites. Purified cMZ-8 is recognized by sera from E. acervulina-infected chickens and induces a

L15 ANSWER 178 OF 209 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 81
AN 1987:490588 BIOSIS
DN BA84:125231
TI MAPPING OF THE GENE CODING FOR EPSTEIN-BARR VIRUS-DETERMINED NUCLEAR ANTIGEN EBNA3 AND ITS TRANSIENT OVEREXPRESSION IN A HUMAN CELL LINE BY USING AN ADENOVIRUS EXPRESSION VECTOR.
AU JOAB I; ROWE D T; BODESCOT M; NICOLAS J-C; FARRELL P J; PERRICAUDET M
CS INST. RECH. SCI. CANCER, CENT. NATL. RECH. SCI., 94800 VILLEJUIF, FR.
SO J VIROL, (1987) 61 (10), 3340-3344.
CODEN: JOVIAM. ISSN: 0022-538X.
FS BA; OLD
LA English
AB The open reading frame which lies within the Epstein-Barr virus (EBV) T2 cDNA isolated by Bodescot et al. (M. Bodescot, O. Brison, and M. Pericaudet, Nucleic Acids Res. 14:2611-2620, 1986) was inserted into a eucaryotic expression vector containing a strong adenovirus promoter. The T2 cDNA contains viral genomic sequences from the short BLRF3 open reading frame fused to the adjacent BERF1 long open reading frame. After transfection of human cells, the **recombinant** plasmid directed the expression of a **140-kilodalton** protein. The expressed protein had the same molecular weight, subcellular localization, and immunological characteristics as the EBV-determined nuclear antigen EBNA3, which is made in lymphocytes latently infected with EBV. Immunoprecipitation of extracts of transfected cells labeled wth [32P]phosphoric acid showed that the EBNA3 protein is phosphorylated.

neutralizing antibodies to SA11 rotavirus.

L15 ANSWER 206 OF 209 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 98
AN 1986:358953 BIOSIS
DN BA82:63427
TI EXPRESSION OF MYCOPLASMA-PNEUMONIAE ANTIGENS IN ESCHERICHIA-COLI.
AU TREVINO L B; HALDENWANG W G; BASEMAN J B
CS UNIV. TEXAS HEALTH SCI. CENTER AT SAN ANTONIO, DEP. MICROBIOLOGY, SAN ANTONIO, TEXAS 78284.
SO INFECT IMMUN, (1986) 53 (1), 129-134.
CODEN: INFIBR. ISSN: 0019-9567.
FS BA; OLD
LA English
AB A genomic library of Mycoplasma pneumoniae was generated by using bacteriophage lambda EMBL3 as the vector. Screening of the library for the expression of M. pneumoniae protein antigens with adsorbed anti-M. pneumoniae serum revealed strong reactivity from a third of those clones which contained mycoplasma DNA inserts. Three of the most highly reactive clones were analyzed in detail and found to synthesize discrete mycoplasma proteins. Two carried overlapping fragments of mycoplasma DNA which encoded a protein that was readily detected in Escherichia coli after infection with **recombinant** bacteriophage. The third clone contained a novel mycoplasma DNA fragment which directed the synthesis of two additional mycoplasma proteins. Further screening of the library with antiserum raised against the major M. pneumoniae adhesin protein P1 (165 kilodaltons [kDa]) yielded one clone which produced an immunologically reactive protein of **140 kDa**. Adsorption of anti-P1 serum by this clone selected a population of antibodies that were reactive with M. pneumoniae adhesin P1 (165 kDa). These results demonstrate that immunologically active M. pneumoniae proteins are

L15 ANSWER 196 OF 209 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 92
AN 1986:338513 BIOSIS
DN BA82:52717
TI EXPRESSION OF THE HUMAN C-FMS PROTO-ONCOGENE PRODUCT COLONY-STIMULATING FACTOR-1 RECEPTOR ON PERIPHERAL BLOOD MONONUCLEAR CELLS AND CHORIOCARCINOMA CELL LINES.
AU RETTENMIER C W; SACCA R; FURMAN W L; ROUSSEL M F; HOLT J T; NIENHUIS A W; STANLEY E R; SHERR C J
CS DEP. OF TUMOR CELL, ST. JUDE CHILDREN'S RES. HOSP., MEMPHIS, TENN. 38105.
SO J CLIN INVEST, (1986) 77 (6), 1740-1746.
CODEN: JCINAO. ISSN: 0021-9738.
FS BA; OLD
LA English
AB The c-fms gene product is related, and possibly identical, to the receptor the mononuclear phagocyte colony stimulating factor, CSF-1. Using antisera to a **recombinant** v-fms-coded polypeptide, glycoproteins encoded by the human c-fms locus were detected in mononuclear cells from normal peripheral blood and in promyelocytic HL-60 cells 24 h after induction of monocytic differentiation with phorbol ester. The **150-kD** human c-fms-coded glycoprotein was expressed at the cell surface, was active as a tyrosine-specific protein kinase in vitro, and shared primary structural features with the product of the feline retroviral v-fms oncogene. A biochemically indistinguishable glycoprotein was detected in human choriocarcinoma cells lines. Like peripheral blood mononuclear cells and phorbol ester-treated HL-60 cells, the choriocarcinoma cells expressed high affinity binding sites for human CSF-1. In addition to serving as a lineage specific growth factor in hematopoiesis, CSF-1, may play a role

Claim 38 (Amended). A [purified protein of the] recombinantly produced *Helicobacter pylori* cytotoxin (CT) polypeptide, wherein the recombinantly produced polypeptide exhibits substantially no toxicity, or substantially reduced toxicity.

Claim 39 (Amended). [The purified protein of claim 38 wherein said protein is] A recombinantly produced fragment of a *Helicobacter pylori* CT polypeptide, wherein the recombinantly produced fragment (i) comprises at least about ten amino acids, (ii) can induce the production of antibodies to *Helicobacter pylori*, and (iii) exhibits substantially no toxicity, or substantially reduced toxicity.

Claim 40 (Amended). A [polypeptide sequence of the *Helicobacter pylori* cytotoxin amino acid sequence set forth in] recombinantly produced *H. pylori* CT polypeptide or fragment thereof comprising SEQ ID NO:3 or a fragment thereof, which polypeptide [sequence] or fragment thereof : (i) comprises at least [five] about ten amino acids of SEQ ID NO:3, (ii) can induce the production of antibodies to *Helicobacter pylori*, and (iii) exhibits substantially no [contribution to] toxicity, or substantially reduced toxicity.

Claim 42 (Amended). The polypeptide [sequence] of claim 40, wherein said [sequence] polypeptide comprises [about five to] at least about fifteen amino acids.

Claim 43 (Amended). A prophylactic or therapeutic vaccine comprising an immunologically effective amount of a *H. pylori* CT polypeptide [sequence of the *Helicobacter pylori* cytotoxin amino acid sequence set forth in] comprising SEQ ID NO:3 or a fragment thereof, which polypeptide [sequence]: (i) comprises at least [five] about ten amino acids of SEQ ID NO:3, (ii) can induce the production of antibodies to *Helicobacter pylori*, and (iii) exhibits substantially no [contribution to] toxicity, or substantially reduced toxicity.

44. The vaccine of claim 43 wherein said sequence comprises at least ten amino acids.

Claim 45 (Amended). The vaccine of claim 43, wherein said [sequence] polypeptide comprises [about five to] at least about fifteen amino acids.

Claim 46 (Amended). The vaccine of claim 43, [which] further [comprises] comprising an immunologically effective amount of a second polypeptide [sequence of the *Helicobacter*] comprising *H. pylori* cytotoxin associated immunodominant (CAI) antigen or a fragment thereof, which second polypeptide [sequence]: (i) comprises at least [five] about ten amino acids, (ii) can induce the production of antibodies to *Helicobacter pylori*, and (iii) exhibits [substantially] no functional contribution to toxicity, or a substantially reduced functional contribution to toxicity.

47. The vaccine of claim 46 wherein said sequence comprises at least ten amino acids.

Claim 48 (Amended). The vaccine of claim 46, wherein said [sequence] second polypeptide comprises [about five to] at least about fifteen amino acids.

Claim 49 (Amended). A method of [preparation of] preparing a prophylactic or therapeutic vaccine [which comprises] comprising bringing into association:

(1) an immunologically effective amount of a [polypeptide sequence of the *Helicobacter pylori* cytotoxin] *H. pylori* CT polypeptide, which polypeptide [sequence]: (i) comprises at least [five] about ten amino acids, (ii) can induce the production of antibodies to *Helicobacter pylori*, and (iii) exhibits substantially no [contribution to] toxicity, or substantially reduced toxicity, and

(2) a pharmaceutically acceptable carrier.

Claim 50 (Amended). The method of claim 49, [which] further [comprises] comprising adding an immunologically effective amount of a second polypeptide [sequence of the *Helicobacter*] comprising *H. pylori* [cytotoxin associated immunodominant (]CAI[)] antigen or fragment thereof [amino acid sequence set forth in SEQ ID NO:5], which second polypeptide [sequence]: (i) comprises at least [five] about ten amino acids, (ii) can induce the production of antibodies to *Helicobacter pylori*, and (iii) exhibits [substantially] no functional contribution to toxicity, or a substantially reduced functional contribution to toxicity.

Claim 52 (New). The method of claim 50, wherein the second polypeptide comprises SEQ ID NO:5, or a fragment thereof, which second polypeptide: (i) comprises at least about ten amino acids, (ii) can induce the production of antibodies to *Helicobacter pylori*, and (iii) exhibits no functional contribution to toxicity, or a substantially reduced functional contribution to toxicity.

Claim 51 (New). A prophylactic or therapeutic vaccine comprising an immunologically effective amount of a recombinantly produced *H. pylori* CT polypeptide, wherein said recombinantly produced polypeptide exhibits substantially no toxicity, or substantially reduced toxicity, and a pharmaceutically acceptable carrier.